

CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the patent application.

- 1) **(ORIGINAL)** A process for the enhanced recovery of recombinant insulin comprising, treating the expression broth/culture medium containing the expressing cells either with water miscible organic solvents and/or with salts and/or with amino acids and/or at different pH conditions, wherein the concentration of the insulin in solution is higher, than had the expression broth or culture medium not been treated with the said conditions.
- 2) **(ORIGINAL)** A process for the enhanced recovery of recombinant insulin comprising, treating the expression broth/culture medium containing the expressing cells either with urea, wherein the concentration of the insulin in solution is higher, than had the expression broth or culture medium not been treated with urea.
- 3) **(ORIGINAL)** A process according to claim 1 where the treatment is carried out from about pH 2.0 to about pH 5.0.
- 4) **(ORIGINAL)** A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with Arginine, at a pH from about 2 to about 4.
- 5) **(ORIGINAL)** A process according to claim 4 wherein the concentration of arginine is from about 1 to about 100 mM.

6) **(ORIGINAL)** A process according to claim 4 wherein the arginine concentration is from about 50 mM to about 100 mM.

7) **(ORIGINAL)** A process according to claim 4 wherein the arginine concentration is from about 70 mM to about 100 mM.

8) **(ORIGINAL)** A process according to claim 4 wherein the arginine concentration is about 100 mM.

9) **(ORIGINAL)** A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with Aspartic acid, at a pH from about 2 to about 4.

10) **(ORIGINAL)** A process according to claim 9 wherein the concentration of aspartic acid is from about 1 mM to about 100 mM.

11) **(ORIGINAL)** A process according to claim 9 wherein the aspartic acid concentration is from about 50 mM to about 100 mM.

12) **(ORIGINAL)** A process according to claim 9 wherein the aspartic acid concentration is from about 70 mM to about 100 mM.

13) **(ORIGINAL)** A process according to claim 9 wherein the aspartic acid concentration is about 100 mM.

14) **(ORIGINAL)** A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with calcium chloride, at a pH from about 2 to about 4.

15) **(ORIGINAL)** A process according to claim 14 wherein the concentration of calcium chloride is from about 1 mM to about 100 mM.

16) **(ORIGINAL)** A process according to claim 14 wherein the calcium chloride concentration is from about 50 mM to about 100 mM.

17) **(ORIGINAL)** A process according to claim 14 wherein the calcium chloride concentration is from about 70 mM to about 100 mM.

18) **(ORIGINAL)** A process according to claim 14 wherein the calcium chloride concentration is about 100mM.

19) **(ORIGINAL)** A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with isopropanol, at a pH from about 2 to about 5.

20) **(ORIGINAL)** A process according to claim 19 where the concentration of isopropanol is from about 1 percent to about 50 percent.

21) **(ORIGINAL)** A process according to claim 19 where the isopropanol concentration is from about 10 percent to about 50 percent.

22) **(ORIGINAL)** A process according to claim 19 where the isopropanol concentration is from about 30 percent to about 50 percent.

23) **(ORIGINAL)** A process according to claim 19 where the isopropanol concentration

is from about 40 percent to about 50 percent.

24) **(ORIGINAL)** A process according to claim 19 where the isopropanol concentration is about 20 percent.

25) **(ORIGINAL)** A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with methanol, at a pH from about 2 to about 5.

26) **(ORIGINAL)** A process according to claim 25 where the concentration of methanol is from about 1 percent to about 50 percent.

27) **(ORIGINAL)** A process according to claim 25 where the methanol concentration is from about 10 percent to about 50 percent.

28) **(ORIGINAL)** A process according to claim 25 where the methanol concentration is from about 30 percent to about 50 percent.

29) **(ORIGINAL)** A process according to claim 25 where the methanol concentration is from about 40 percent to about 50 percent.

30) **(ORIGINAL)** A process according to claim 25 where the methanol concentration is about 20 percent.

31) **(ORIGINAL)** A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with ethanol, at a pH from about 2 to about 5.

32) **(ORIGINAL)** A process according to claim 31 where the concentration of ethanol is from about 1 percent to about 50 percent.

33) **(ORIGINAL)** A process according to claim 31 where the ethanol concentration is from about 10 percent to about 50 percent.

34) **(ORIGINAL)** A process according to claim 31 where the ethanol concentration is from about 30 percent to about 50 percent.

35) **(ORIGINAL)** A process according to claim 31 where the ethanol concentration is from about 40 percent to about 50 percent.

36) **(ORIGINAL)** A process according to claim 31 where the ethanol concentration is about 20 percent.

37) **(ORIGINAL)** A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with tertiary-butanol, at a pH from about 2 to about 5.

38) **(ORIGINAL)** A process according to claim 37 where the concentration of tertiary-butanol is from about 1 percent to about 50 percent.

39) **(ORIGINAL)** A process according to claim 37 where the tertiary-butanol concentration is from about 10 percent to about 50 percent.

40) **(ORIGINAL)** A process according to claim 37 where the tertiary-butanol concentration is from about 30 percent to about 50 percent.

41) **(ORIGINAL)** A process according to claim 37 where the tertiary-butanol concentration is from about 40 percent to about 50 percent.

42) **(ORIGINAL)** A process according to claim 37 where the tertiary-butanol concentration is about 20 percent.

43) **(ORIGINAL)** A process as in claim 1 comprising, treatment of the expressionbroth/culture medium containing the expressing cells with acetonitrile, at a pH from about 2 to about 5.

44) **(ORIGINAL)** A process according to claim 43 where the concentration of acetonitrile is from about 1 percent to about 50 percent.

45) **(ORIGINAL)** A process according to claim 43 where the acetonitrile concentration is from about 10 percent to about 50 percent.

46) **(ORIGINAL)** A process according to claim 43 where the acetonitrile concentration is from about 30 percent to about 50 percent.

47) **(ORIGINAL)** A process according to claim 43 where the acetonitrile concentration is from about 40 percent to about 50 percent.

48) **(ORIGINAL)** A process according to claim 43 where the acetonitrile concentration is about 20 percent.

49) **(ORIGINAL)** A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with dimethylformamide.

50) **(ORIGINAL)** A process according to claim 49 where the concentration of dimethylformamide is from about 1 percent to about 50 percent.

51) **(ORIGINAL)** A process according to claim 49 where the dimethylformamide concentration is from about 10 percent to about 50 percent.

52) **(ORIGINAL)** A process according to claim 49 where the dimethylformamide concentration is from about 30 percent to about 50 percent.

53) **(ORIGINAL)** A process according to claim 49 where the dimethylformamide concentration is from about 40 percent to about 50 percent.

54) **(ORIGINAL)** A process according to claim 49 where the dimethylformamide concentration is about 20 percent.

55) **(ORIGINAL)** A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with dimethylsulfoxide, at a pH from about 2 to about 5.

56) **(ORIGINAL)** A process according to claim 55 where the concentration of dimethylsulfoxide is from about 1 percent to about 50 percent.

57) **(ORIGINAL)** A process according to claim 55 where the dimethylsulfoxide concentration is from about 10 percent to about 50 percent.

58) **(ORIGINAL)** A process according to claim 55 where the dimethylsulfoxide concentration is from about 30 percent to about 50 percent.

59) **(ORIGINAL)** A process according to claim 55 where the dimethylsulfoxide concentration is from about 40 percent to about 50 percent.

60) **(ORIGINAL)** A process according to claim 55 where the dimethylsulfoxide concentration is about 20 percent.

61) **(ORIGINAL)** A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with ethylene glycol, at a pH from about 2 to about 5.

62) **(ORIGINAL)** A process according to claim 61 where the concentration of ethylene glycol is from about 1 percent to about 50 percent.

63) **(ORIGINAL)** A process according to claim 61 where the ethylene glycol concentration is from about 10 percent to about 50 percent.

64) **(ORIGINAL)** A process according to claim 61 where the ethylene glycol concentration is from about 30 percent to about 50 percent.

65) **(ORIGINAL)** A process according to claim 61 where the ethylene glycol concentration is from about 40 percent to about 50 percent.

66) **(ORIGINAL)** A process according to claim 61 where the ethylene glycol concentration is about 20 percent.

67) **(ORIGINAL)** A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with propylene glycol, at a pH from about 2 to about 5.

68) **(ORIGINAL)** A process according to claim 67 where the concentration of ethylene glycol is from about 1 percent to about 50 percent.

69) **(ORIGINAL)** A process according to claim 67 where the propylene glycol concentration is from about 10 percent to about 50 percent.

70) **(ORIGINAL)** A process according to claim 67 where the propylene glycol concentration is from about 30 percent to about 50 percent.

71) **(ORIGINAL)** A process according to claim 67 where the propylene glycol concentration is from about 40 percent to about 50 percent.

72) **(ORIGINAL)** A process according to claim 67 where the propylene glycol concentration is about 20 percent.

73) **(ORIGINAL)** A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with acetic acid, at a pH from about 2 to about 5.

74) **(ORIGINAL)** A process according to claim 73 where the concentration of acetic acid is from about 1 percent to about 50 percent.

75) **(ORIGINAL)** A process according to claim 73 where the acetic acid concentration is from about 10 percent to about 50 percent.

76) **(ORIGINAL)** A process according to claim 73 where the acetic acid concentration is from about 30 percent to about 50 percent.

77) **(ORIGINAL)** A process according to claim 73 where the acetic acid concentration is from about 40 percent to about 50 percent.

78) **(ORIGINAL)** A process according to claim 73 where the acetic acid concentration is about 20 percent.

79) **(ORIGINAL)** A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with dioxan, at a pH of about 2 to about 5.

80) **(ORIGINAL)** A process according to claim 79 where the concentration of dioxan is from about 1 percent to about 20 percent.

81) **(ORIGINAL)** A process according to claim 79 where the dioxan concentration is from about 10 percent to about 20 percent.

82) **(ORIGINAL)** A process according to claim 79 where the dioxan concentration is from about 15 percent to about 20 percent.

83) **(ORIGINAL)** A process according to claim 79 where the dioxan concentration is about 20 percent.

84) **(ORIGINAL)** A process as in claim 2 comprising, treatment of the expression broth/culture medium containing the expressing cells with urea, at about pH of about 2 to about 5.

85) **(ORIGINAL)** A process according to claim 84 where the concentration of urea is

from about 1M to about 6M.

86) **(ORIGINAL)** A process according to claim 84 where the urea concentration is from about 3M to about 6M.

87) **(ORIGINAL)** A process according to claim 84 where the urea concentration is 6M.

88) **(ORIGINAL)** A process for the enhanced recovery of recombinantly expressed insulin into solution, comprising, treatment of the expression broth/culture medium containing the expressing cells with isopropanol at pH about 2, followed by removal of the supernatant, followed by extraction (s).

89) **(ORIGINAL)** A process for the enhanced recovery of recombinantly expressed insulin into solution according to claim 88, comprising at least two extraction steps with isopropanol, of the expression broth/culture medium containing the expressing cells, at pH about 2.

90) **(ORIGINAL)** A process according to claim 89 wherein the concentration of isopropanol used in each step is same or different.

91) **(ORIGINAL)** A process according to claim 90 wherein the concentration of isopropanol used in each step is different.

92) **(ORIGINAL)** A process according to claim 90 wherein the concentration of isopropanol used in each step is same.

93) **(ORIGINAL)** A process according to claim 92 wherein the concentration of isopropanol used is, from about 1 percent to about 50 percent.

94) **(ORIGINAL)** A process according to claim 92 wherein the isopropanol concentration is from about 10 percent to about 50 percent.

95) **(ORIGINAL)** A process according to claim 92 wherein the isopropanol concentration is from about 30 percent to about 50 percent.

96) **(ORIGINAL)** A process according to claim 92 wherein the isopropanol concentration is from about 40 percent to about 50 percent.

97) **(ORIGINAL)** A process according to claim 92 wherein the isopropanol concentration is about 20 percent.

98) **(ORIGINAL)** A process for the enhanced recovery of recombinantly expressed insulin into solution, comprising of sequential extraction of the expression broth/culture medium containing the expressing cells with at least two of following steps, but not necessarily in the same order: a) citrate buffer at about pH 2 to about pH 5 b) about 0.2 M to about 1.0 M sodium chloride at about pH 2 to about pH 5 c) about 10 percent to about 50 percent isopropanol at about pH 2 to about pH 5 d) about 1M to about 5M urea at about pH 2 e) about 1M to about 5M urea at about pH 4.

99) **(ORIGINAL)** A process for the isolation and purification of insulin from recombinant sources consisting of subjecting the expression broth/culture medium containing the expressing cells to chromatography in an expanded mode.

100) **(ORIGINAL)** A process as in claim 99 wherein the chromatography is ion exchange chromatography.

101) **(ORIGINAL)** A process according to claim 100 wherein the ion exchange chromatography is cation exchange chromatography.

102) **(ORIGINAL)** A process according to claim 101 in which the fermentationbroth/culture medium containing the expressing cells is diluted with buffer at about pH 3, then fed to column packed with Streamline-SP cation exchange resin, followed by washing with buffer at about pH 4 and then followed by elution with buffer at about pH 7.5.

103) **(ORIGINAL)** A process according to claim 101, wherein the dilution buffer is citrate buffer, wash buffer is citrate buffer.

104) **(ORIGINAL)** A process according to claim 101, wherein the elution buffer is Tris HCl buffer

105) **(ORIGINAL)** A process according to claim 101, wherein the dilution buffer is citrate buffer, wash buffer is citrate buffer and the elution buffer is TrisHCl buffer

106-129 **(CANCELED)**

130. (NEW) A process for enhanced recovery of recombinant insulin, the process comprising:

- (a) treating an expression broth/culture medium containing the expressing cells with a water miscible organic solvent; and
- (b) isolating the insulin from the treated broth/culture by using chromatography technique in an expanded bed mode.

131. (NEW) The process of claim 130, wherein the water miscible organic solvent is

selected from the group consisting of methanol, ethanol, isopropanol, acetic acid, dimethylformamide, dimethylsulfoxide, acetonitrile, dioxan, ethylene glycol, and propylene glycol.

132. (NEW) The process of claim 131, wherein the water miscible organic solvent is isopropanol.

133. (NEW) The process of claim 130, wherein the water miscible organic solvent is used at a concentration range from about 10% (v/v) to about 40% (v/v).

134. (NEW) The process of claim 133, wherein the water miscible organic solvent is used at a concentration of about 20% (v/v).

135. (NEW) The process of claim 130, wherein the water miscible organic solvent may further include additives.

136. (NEW) The process of claim 135, wherein the additives comprise one or more of sodium chloride, calcium chloride, arginine, aspartic acid, urea, guanidinium hydrochloride, or polyethylene glycol.

137. (NEW) The process of claim 130, wherein the process is carried out at a pH range from about 2 to about 5.

138. (NEW) The process of claim 137, wherein the process is carried out at a pH of about 3.

139. (NEW) The process of claim 130, wherein the chromatography is an ion exchange chromatography.

140. (NEW) The process of claim 139, wherein the ion exchange chromatography is a cation exchange chromatography.
141. (NEW) The process of claim 130, wherein the recombinant source expressing the insulin is yeast.
142. (NEW) The process of claim 141, wherein the yeast comprises genera *Hansenula*, *Saccharomyces*, *Pichia*, or *Kluyveromyces*.